

Monitoring of Airborne Fungi in a Hospital Unit

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ABSTRACT

Background: Nosocomial infections are caused mainly by airborne pathogens found in health care facilities and their surroundings. Fungi are ubiquitous in environment and are considered critical pathogens of hospital-associated infections. **Objective:** The aim of this study was to evaluate the concentrations of fungal species in the air of a tertiary care hospital. **Methods:** The air samples were collected by open plate technique once after 3 months from indoor and outdoor area of different wards and operation theaters over different periods of the year. In total, 160 air samples were cultured into Sabouraud dextrose agar and Blood agar plates and incubated at 25°C for 7-10 days. Fungal species were identified by macroscopic and microscopic features. The number of airborne fungi was presented in colony-forming unit per cubic meter (CFU/m³). **Results:** Four hundred sixty-nine colonies were isolated in a year. The highest fungal CFU was isolated from female surgery ward and least from operation theaters. The most isolated fungi were *Aspergillus* spp. (29.42%), followed by *Curvularia* spp. (15.77%), *Alternaria* spp. (14.28%), *Scedosporium apiospermum* (10.23%), *Penicillium* spp. (8.95%), *Cladophialophora* spp. (7.46%), *Paecilomyces* spp. (6.82%). The yeast spp. isolated from indoor and outdoor samples were 7.09% and 4.77%, respectively. **Conclusions:** Different fungal species may have different pathogenicities and body resistance is the key against fungal infections. Therefore, the need of the hour is to monitor the indoor air to prevent nosocomial infections.

Keywords: Airborne fungal spores, *Aspergillus*, indoor air, nosocomial infections

Physical, chemical and biological agents of the indoor and outdoor environment can affect public health.¹ Indoor air quality in hospitals is a concern because it contains a wide range of infectious airborne microorganisms that may cause hospital infections.^{2,3} Immunocompromised patients are more prone to nosocomial infections, due to reduction in their defensive ability—whether due to cancer, hemolymphoproliferative diseases or human immunodeficiency virus (HIV) infection,^{4,7} medical therapy or organ transplantation. Bioaerosols in the hospital air are one of the potential sources of infection.⁸ Fungi and bacteria are the major types of bioaerosols present in hospital environments.^{9,10} Fungal pollutants in indoor environment depend on many factors such as: temperature, moisture, ventilation and organic matter present in building materials. Also, outdoor fungal spores may be transmitted through visitors,

patients and air conditioning.¹⁰ Airborne micro-fungi in indoor hospital environments are mainly formed by filamentous fungi that belong to the *Aspergillus* species (spp.), Mucorales (*Rhizopus* spp.), *Fusarium* spp., *Cladosporium* spp., *Paecilomyces* spp., *Scedosporium* spp., *Penicillium* spp., *Scopulariopsis* spp., *Pseudallescheria boydii*, *Sporothrix* spp. and *Acremonium* spp.^{8,11,12} Yeast isolates have also been found that belong to the genus of *Candida*, *Trichosporon*, *Rhodotorula*, *Saccharomyces* and *Cryptococcus*.^{11,13-15} The evaluation of density and diversity of bioaerosols in the hospital can be a good indicator of the cleanliness of these environments.³

The aim of this study was to assess the fungal aerosols in indoor and outdoor environment of hospital, which will be helpful in future to reduce fungal hospital-acquired infection (HAI) rates in immune compromised patients.

MATERIAL AND METHODS

A prospective surveillance study was conducted in the Dept. of Microbiology of a teaching hospital for a period of 1 year (April 2018 to March 2019) to evaluate the airborne fungal contamination in the hospital by Settled Plate method. Prior to the study, clearance from hospital ethical committee was taken. Both indoor and outdoor samples were collected from Surgery, Medicine, Obstetrics and Gynecology wards,

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Labor room, Intensive care unit and Operation theaters over different periods of the year. Outdoor samples were collected as control for comparison of indoor microbial loads.

The air samples were collected by open plate method (passive sampling method).¹⁶ The two standard petri dishes containing culture media of Sabouraud dextrose agar (SDA) with chloramphenicol and Blood agar (BA) were put at a height of 100-150 cm above the ground level during sampling for 20 minutes¹⁷ to collect fungal particles. This procedure was repeated at an interval of 3 months. On the whole 160 (BA [80] and SDA [80]) plates were used for indoor and outdoor samples over 1 year period.

After exposure, the plates were placed in BOD incubators at 25°C for 7-10 days and monitored daily. The fungal colonies were identified on the basis of macroscopic and microscopic morphology. Macroscopically, the surface, texture and pigment production by fungi were noted. The presence of specific reproductive structures, presence/absence of conidia and their size, shape and structure of conidia, septation in mycelium were noted microscopically by seeing the lactophenol cotton blue wet mount preparation. Data was statically analyzed.

RESULTS

In this study, total 469 fungal colonies were isolated in a year. Out of these, 155 (33%) colonies were isolated from indoor samples and 314 (67%) from outdoor samples. The highest fungal colony-forming unit (CFU) were isolated from female surgery ward (Table 1) and least from operation theaters. The most isolated fungi were *Aspergillus* spp. (29.42%), followed by *Curvularia* spp. (15.77%), *Alternaria* spp. (14.28%), *Scedosporium apiospermum* (10.23%), *Penicillium* spp. (8.95%), *Cladophialophora* spp. (7.46%), *Paecilomyces* spp. (6.82%) (Table 2). The dominant fungi isolated from surgery and medicine wards were *Penicillium*, *Aspergillus* spp. and *S. apiospermum*. The yeast spp. isolated from indoor and outdoor samples were 7.09% and 4.77%, respectively. The mean of different fungal genera isolated from indoor and outdoor plates were 1.93 and 3.9 fungus/plate, respectively.

DISCUSSION

In the present study, the concentration and distribution of fungi in the indoor air and outdoor air were monitored over 1 year. The distribution of fungi was quite different between indoor and outdoor environment. Pattern of indoor and outdoor isolates was similar. Fungal loads in indoor air were less than that of the outdoor air. Whenever outdoor microbial loads increased, there was

Table 1. Concentration of Fungal Population in Indoor and Outdoor Air of Different Wards and Operation Theaters in the Hospital

Sample place	CFU	Mean	Standard deviation
Wards (Indoor)			
Surgery (Male)	24	3	1.5
Surgery (Female)	28	3.5	1.4
OBG	22	2.7	1.66
Medicine (Male)	25	3.12	1.72
Medicine (Female)	22	2.7	1.66
Wards (Outdoor)			
Surgery (Male)	48	6	2.5
Surgery (Female)	40	5	1.06
OBG	42	5.25	3.23
Medicine (Male)	45	5.06	1.48
Medicine (Female)	53	6.62	2.38
Labor room (Indoor)	7	0.87	0.53
Labor room (Outdoor)	13	1.62	0.91
ICU (Indoor)	15	1.87	0.33
ICU (Outdoor)	55	6.8	1.45
NICU (Indoor)	7	0.87	0.53
NICU (Outdoor)	10	1.25	0.7
Operation theater (OT)			
Main OT (Indoor)	3	0.37	0.5
Main OT (Outdoor)	7	0.87	0.53
Gyne OT (Indoor)	2	0.25	0.34
Gyne OT (Outdoor)	1	0.12	0.34

a corresponding increase in microbial loads of indoor air. This indicates that indoor microflora is influenced by outdoor environmental conditions. Problems of indoor air quality are recognized as important risk factors for human health and were documented previously also by different workers.¹⁸⁻²⁰ Indoor air is important also because populations spend a substantial fraction of time within buildings. Microbial pollution involves hundreds of species of bacteria and fungi that grow indoors when sufficient moisture is available. Exposure to microbial contaminants is clinically associated with respiratory symptoms, allergies, asthma and immunological reactions.²¹ The amount of fungi after entering from the outdoor environment tends to increase in the presence of favorable conditions in the indoor environment and can cause health problems in both immunodeficient as well as healthy people.²² Airborne fungal spores are very important agents in nosocomial infection and respiratory diseases and their effect on human health

Table 2. Percentage of Different Fungi Isolated from Indoor and Outdoor Environment of Wards and Operation Theaters

Fungus	Indoor fungi isolated (%)	Outdoor fungi isolated (%)	Mean (%)
<i>Aspergillus niger</i>	23 (14.83)	42 (13.37)	32.5 (13.85)
<i>Aspergillus flavus</i>	13 (8.38)	28 (8.9)	20.5 (8.74)
<i>Aspergillus fumigatus</i>	08 (5.16)	24 (7.64)	16 (6.82)
<i>Curvularia</i> spp.	27 (17.41)	47 (14.96)	37 (15.77)
<i>Alternaria</i> spp.	24 (15.48)	43 (13.69)	33.5 (14.28)
<i>Scedosporium apiospermum</i>	15 (9.67)	33 (10.5)	24 (10.23)
<i>Penicillium</i> spp.	14 (9.03)	28 (8.91)	21 (8.95)
<i>Cladophialophora</i> spp.	10 (6.45)	25 (7.96)	17.5 (7.46)
<i>Paecilomyces</i> spp.	08 (5.16)	24 (7.64)	16 (6.82)
<i>Rhizopus</i> spp.	2 (1.3)	05 (1.59)	3.5 (1.49)
Yeast spp.	11 (7.09)	15 (4.77)	13 (5.54)
Total colonies isolated	155	314	-

is linked with their genera, species and concentrations in air.^{16,17} Many studies have shown the relationship between HAI and the existence of microorganisms in hospital environments, including in the air of wards.^{2,23}

In the present study, 33% fungal colonies were isolated from indoor air samples, whereas other workers reported much higher rates of 62.03% and 69.11 CFU/m³.^{23,24} No significant seasonal variation was seen in the distribution of the fungi with regards to the average spore counts. In this study, among filamentous fungi, *Aspergillus* spp. were the most dominant fungal isolates followed by *Curvularia* spp. and *Alternaria* spp. *Aspergillus* spp. was found in high numbers in indoor air samples. *Aspergillus* spp. that can grow indoors include *Aspergillus fumigatus* and *Aspergillus flavus* and can cause nosocomial infections,²⁵ allergic bronchopulmonary aspergillosis and sinusitis. Aspergillosis can occur in immunocompromised hosts or as a secondary infection, following inhalation of fungal spores or the toxins produced by them. Symptoms include persistent cold, watery eyes, prolonged muscle cramps and joint pain, etc. Chronic asthmatics may progress to have their bronchial passages colonized by either *A. fumigatus*, *Bipolaris hawaiiensis* or *Wangiella dermatitidis*.¹⁹ Constant allergic response maintains the fungal colonization.¹⁹ *Aspergillus* species can cause invasive Aspergillosis and produce mycotoxins which are known to be carcinogens. Although there are no strict numerical guidelines for determining the level of fungal contamination in hospital air, the national guidelines of the United Kingdom for prevention of nosocomial aspergillosis for interpretation of the fungal spore burden state that the fungal spores in air should be ≤ 5 conidia/m³ in the absence of air

filtration.²⁶ Other fungal spores also have the ability to cause allergies as well as other respiratory diseases and hypersensitivity reactions not only in immune suppressed patients but also in healthy individuals.

Hence, more attention should be given to safeguard indoor environments; otherwise the growth of pathogenic microorganisms can cause toxigenic health hazards.²⁷ The present study showed that *Curvularia* spp. and *Alternaria* spp. were also predominant fungi isolated from indoor. Other workers also reported *Aspergillus*, *Penicillium* and *Alternaria* as the most frequently isolated fungal genera in indoor.^{16,28} It seems that different geographic locations can influence the dominant fungal agents. *Curvularia* spp. is responsible for nosocomial dialysis-related peritonitis and post-surgical endocarditis, whereas *Alternaria* spp. can cause deep infections in the immunocompromised patients.²⁹ Our results showed that almost all of the wards were polluted by various fungi.

CONCLUSION

Different fungal species may have different pathogenicities and body resistance is the key against fungal infection. Therefore, it needs in-depth investigation of aerosol fungal pathogenicity and body immunity to determine harms of fungal aerosols in people.

Regular surveillance and stringent measures including air disinfection system, ventilation systems, using the high-efficiency particulate air (HEPA) filters for high-risk wards, closing the windows, control entry and exit doors, control or totally eliminate flowers taken by the patients' visitors are necessary to reduce mold spores.

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